TECHNICAL MANUSCRIPT 86

COCCIDIOIDOMYCOSIS: STUDIES OF CANINE VACCINATION AND THERAPY

OCTOBER 1963

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 86

COCCIDIOIDOMYCOSIS: STUDIES OF CANINE VACCINATION AND THERAPY

M. W. Castleberry
J. L. Converse
J. T. Sinski
E. P. Lowe
S. P. Pakes
J. E. Del Favero

Pathology Division
DIRECTOR OF MEDICAL RESEARCH

Project 1C022301A070

October 1963

A portion of the work reported here was performed under Project 4B11-02-068, "Acrobiological Research," Task -03, "Pathogenesis of BW Aerosol-Induced Infection." The expenditure order was 2073. This material was originally submitted as manuscript 5195.

ABSTRACT

A three-phase study of vaccination and antibiotic therapy in experimental pulmonary coccidioidomycosis of dogs was made to determine: (a) the efficacy of various routes of inoculation of a formalin-killed, arthrospore vaccine; (b) the combined effects of vaccination and oral Amphotericin B therapy administered immediately following respiratory exposure to Coccidioides immitis; and (c) renal damage or nephrotoxicity resulting from oral Amphotericin B therapy. Neither of the pulmonary routes of vaccination (aerosol or intratracheal) provided protection against a subsequent respiratory challenge of approximately 80,000 C. immitis arthrospores, either singly or in combination with oral Amphotericin B therapy (150 milligrams per day for 20 days following challenge), nor did subcutaneous vaccination or therapy alone. However, eight of twelve dogs receiving both subcutaneous vaccination and therapy completely resisted the respiratory challenge; the remaining four exhibited very minimal, self-contained disease. Histopathological examination revealed no renal damage or nephrotoxicity in any of the dogs receiving Amphotericin B therapy (total dose in excess of three grams); their blood urea nitrogen levels remained within normal limits.

CONTENTS

FIGURES

TABLES

I. INTRODUCTION

A number of studies have been made to evaluate the efficacy of nonviable vaccines against experimental coccidioidomycosis in laboratory animals. Negroni et al[1] and Vogel et al[2] used guinea pigs for their studies. Friedman and Smith,[3] Levine et al,[4,5] Converse et al[6] and Kong et al[7] used mice. Levine et al[8] used monkeys. These investigators found that, although survival time of the immunized animals could be extended by the vaccines used, the majority of infected animals harbored viable Coccidioides immitis organisms for long periods of time after challenge.

Campbell and Hill[8] in their studies of therapy in experimental coccidioidomycosis of mice found that the presolubilized form of Amphotericin B (Fungizone, E. R. Squibb and Sons) for intravenous use was more readily absorbed from the gastrointestinal tract than was the preparation made for oral use. The drug was well tolerated and demonstrated no apparent clinical side effects. These investigators, by mixing the Amphotericin B in the drinking water, were able to prolong survival in mice challenged with C. immitis.

The purpose of this study was to compare, in the dog, (a) the efficacy of a nonviable vaccine administered via the pulmonary route with that administered subcutaneously, and (b) to determine the degree of protection afforded by the vaccinations in combination with Amphotericin B therapy.

II. MATERIAL AND METHODS

The vaccine was a suspension in normal saline of formalin-killed, washed arthrospores of the Silveira strain of C. immitis. A spore concentration of forty milligrams per millliliter was used for the aerosol vaccinations. Eight milligrams per millliliter were used for the subcutaneous and intratracheal vaccinations.

Pulmonary vaccination was accomplished by inhalation of the aerosolized vaccine or by intratracheal instillation. A plastic box with a volume of two cubic feet was used as an aerosol chamber. It was equipped with an air filter to permit maintenance of normal air pressure. In addition, two five-inch port holes were cut, one in one side and the other in one end of the box. These were covered with slit rubber diaphragms. A small hole was drilled in the opposite end to permit insertion of the nozzle of a DeVilbiss No. 15 nebulizer. Two dogs at a time were held in place for ten minutes with their muzzles protruding into the box through the diaphragms. Four milliliters of the vaccine were aerosolized into the box over a period of approximately three minutes.

The intratracheal vaccinations were accomplished by first tranquilizing the animals with Sparine (Promazine hydrochloride, Wyeth Lab. Inc.) and then laying them on their backs with their head extended.* One hand of the assistant was placed over the mouth and muzzle. One milliliter of the vaccine (8 milligrams per milliliter) was then injected intratracheally about three centimeters below the larynx with a two-milliliter hypodermic syringe equipped with a 3/4-inch 20-gauge needle. The animal was immediately lifted with the head up and the airway occluded momentarily. Removal of the hand from the muzzle brought instant inspiration of the material deposited in the trachea.

The subcutaneous vaccinations were made by injecting one milliliter of the vaccine (8 mg/ml) in the lateral thorax just behind the scapula.

Two weeks later all dogs were revaccinated as before. One month after the second vaccination, all animals except the controls were challenged via the respiratory route with a calculated average inhaled dose of 80,000 viable arthrospores of strain Silveira. The organisms were grown on agar plates at 34°C until arthrospores had formed. The plates were desiccated and the arthrospores collected as dry powder with a vacuum apparatus.

The challenge aerosol was generated by forcing compressed air into a diaphragm-covered tube containing the powdered arthrospores. The force released as the diaphragm ruptured disseminated the arthrospores throughout the 6200-liter aerosol test chamber. This chamber has been described by Wolfe.[10]

The inhaled challenge dose was calculated from the spore concentration of the cloud, the average volume of canine lungs, the rate of canine respiration, and the time of exposure. Cloud concentration was determined by drawing a measured amount of the aerosol through an in-line filter-paper sampler and making a mycological plate count of appropriate dilutions of the contents of the filter disc.

Immediately following the aerosol challenge, each dog receiving treatment was given 150 milligrams of Amphotericin B dissolved in distilled drinking water. This treatment was repeated daily for 20 days.

Eight weeks following challenge, the dogs were sacrificed with an overdose of Nembutal Veterinary (Abbott Laboratories), administered intravenously. The animals were necropsied and the tissues fixed in 10 per cent buffered formalin, embedded in paraffin, sectioned and stained. The Giemsa, Gomori methenamine silver, and Ziehl-Neelsen acid-fast stains were used.

---

* In conducting the research reported here, the investigators adhered to "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

Thirty-two mature, mixed-breed dogs of both sexes weighing 7 to 10 kilograms each were used in this experiment. The protocol for their therapy and/or vaccination was as follows:

	Challenged		Not Challenged	
	Treated	Not Treated	Treated	Not Treated
Aerosol-Vaccinated	3	3		2
Subcutaneously Vaccinated	3	3		2
Intratracheally Vaccinated	3	3		2
Not Vaccinated	3	3	2	

III. RESULTS

As shown in Table I, neither of the pulmonary routes of vaccination provided protection alone or in combination with Amphotericin B, nor was protection provided by the Amphotericin B alone. Protection was noted only in the subcutaneously vaccinated and treated animals. One dog in this group, L38, had occasional, small, solitary granulomas in the kidney, pancreas, and heart. These were not attributed to coccidioidomycosis. Four dogs in the other groups demonstrated similar lesions in the kidney. One of these, 31H, showed evidence of visceral larval migrans.

Several dogs in the subcutaneously vaccinated group developed sterile abcesses at the vaccination site. Dog 6L9, presumably vaccinated intratracheally, developed a similar abcess to the right of the trachea following his first vaccination.

The eight control dogs in this experiment were without histopathological interest.

The promising results obtained from the subcutaneously vaccinated and treated dogs led to a repetition of this portion of the experiment. All the conditions of the first experiment were repeated except that nine dogs were subcutaneously vaccinated instead of three as before. These nine, plus four additional unvaccinated, untreated dogs, were then challenged via the respiratory route with an average inhaled dose of 7000 arthrospores. Amphotericin B (150 milligrams per day) was again dissolved in distilled water, but for this experiment was administered as a split dose twice daily by mixing it with the animal's food. In both experiments, close attention was given to assure the dog's full daily receipt of drug. No vomiting, diarrhea, or other untoward reactions were ever noted. Two unchallenged untreated dogs served as environmental room controls.

TABLE I. EFFECT OF ROUTE OF VACCINATION AND AMPHOTERICIN B ON THE DEVELOPMENT OF PULMONARY COCCIDIOIDOMYCOSIS IN DOGS EXPOSED TO AEROSOLS OF C. IMMITIS

Vaccination Route	Treated		Not Treated	
	Animal	Results	Animal	Results
Aerosol	90L	+++	74L	++++
	0K2	++	3M4	+++
	2M9	++	9K6	+
Subcutaneous	L38	0	3K7	+
	L73	0	L44	+++
	L32	0	2K0	++
Intratracheal	4M8	+	M98	++
	7M1	+	6L9	0
	99K	++	31M	+++
Not Vaccinated	9N2	++	35N	+++
	31H	+++	L40	+
	M52	++	41N	+++

\+ = Minimal
++ = Moderate
+++ = Severe, no dissemination
++++ = Severe, dissemination

Ten weeks after challenge, all animals were necropsied. The results of this experiment are shown in Table II. In striking contrast to the severe disease in the control animals, only four of the nine dogs receiving vaccination and therapy demonstrated any histopathological changes. These were very minimal and indicative of self-contained disease.

A comparison of the gross and histopathological pulmonary changes noted in these studies is shown in Figures 1 through 6.

IV. DISCUSSION

Hitchner and Reising[11] demonstrated the feasibility of imparting immunity by inhalation of aerosolized attenuated microorganisms. As pointed out by Aleksandrov and his associates,[12] one of the principal advantages of this method is the ease and rapidity of vaccinating large groups. Eigelsbach and co-workers[13] found evidence that aerogenic vaccination with attenuated Pasteurella tularensis afforded perhaps greater immunity against tularemia than did the dermal route.

The results obtained in our study indicate that the immune mechanism was not sufficiently stimulated by inhalation of killed arthrospores to produce an apparent immunity. It is postulated that the lung clearance mechanism disposed of the arthrospores as nonviable particulate matter in sufficient quantity to prevent an apparent response.

No explanation is offered for the sterile abcesses that occurred at the injection site in the subcutaneously vaccinated dogs. This reaction was subsequently greatly reduced, but not entirely eliminated, by dividing the dose and administering it in separate sites.

The nonvaccinated but treated dogs were surprising in their positive response to challenge. Previous mouse Amphotericin B therapy studies in these laboratories (unpublished) had substantiated the results of Campbell and Hill[9] and it had been believed that the dogs would at most show only minimal response to challenge.

Bell et al[14] have reported (in an addendum) the possible association of human and canine renal tubular damage following intravenous administration of the drug. Sanford et al[15] described a nephrocalcinosis and marked morphological changes in three renal biopsies of human patients, with distinct renal functional deficits being noted in all patients so treated. However, no histological evidence of renal damage attributable to the use of Amphotericin B was seen in the two drug control dogs nor in any of the animals receiving treatment. A slight increase in the blood urea nitrogen values of the drug-control dogs were noted during and shortly beyond the test period.

TABLE II. EFFECT OF SUBCUTANEOUS VACCINATION AND AMPHOTERICIN B ON THE DEVELOPMENT OF PULMONARY COCCIDIOIDOMYCOSIS IN DOGS EXPOSED TO AEROSOLS OF C. IMMITIS

Vaccinated - Treated		Not Vaccinated - Not Treated	
Animal	Results	Animal	Results
T80	+		
73S	+		
51S	0		
T19	0		
N70	+		
07T	0	723	+++
OR1	+	781	++
T41	0	R99	++++
OR8	0	T07	++++

\+ = Minimal
++ = Moderate
+++ = Severe, no dissemination
++++ = Severe, dissemination

Figure 2. Lungs from Dog Subcutaneously Vaccinated and not Treated.

Figure 1. Lungs from Dog Subcutaneously Vaccinated and Treated.

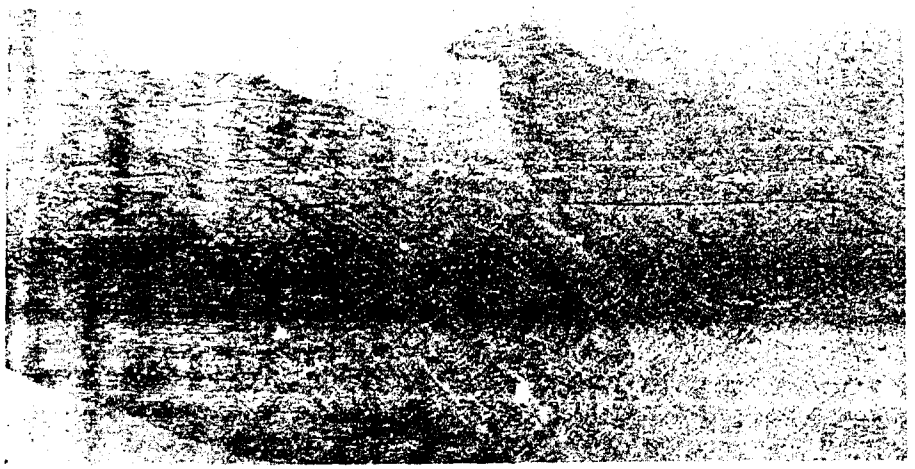

Figure 3. Slide Section, Negative Lungs (1.5X).

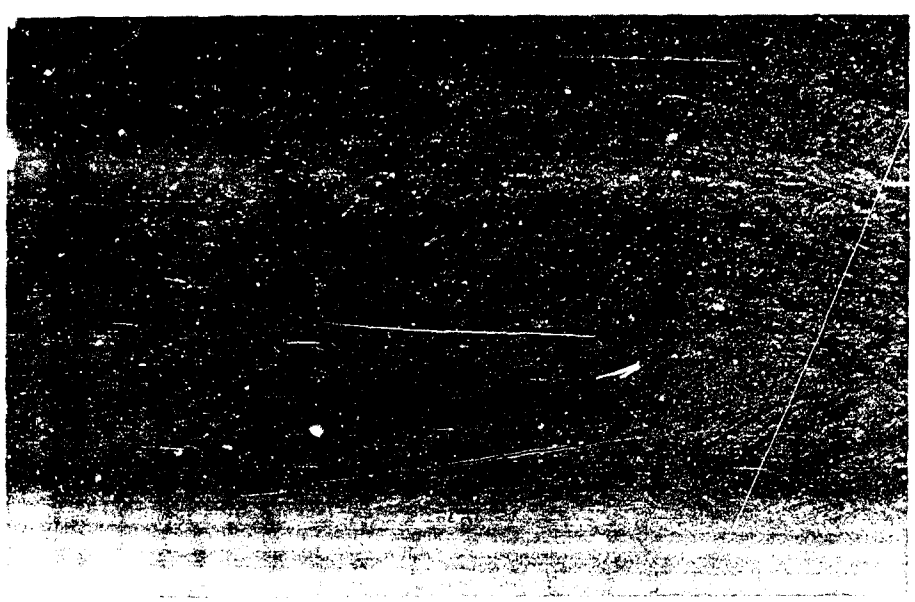

Figure 4. Slide Section, Lung Minimally Affected (1.5X).

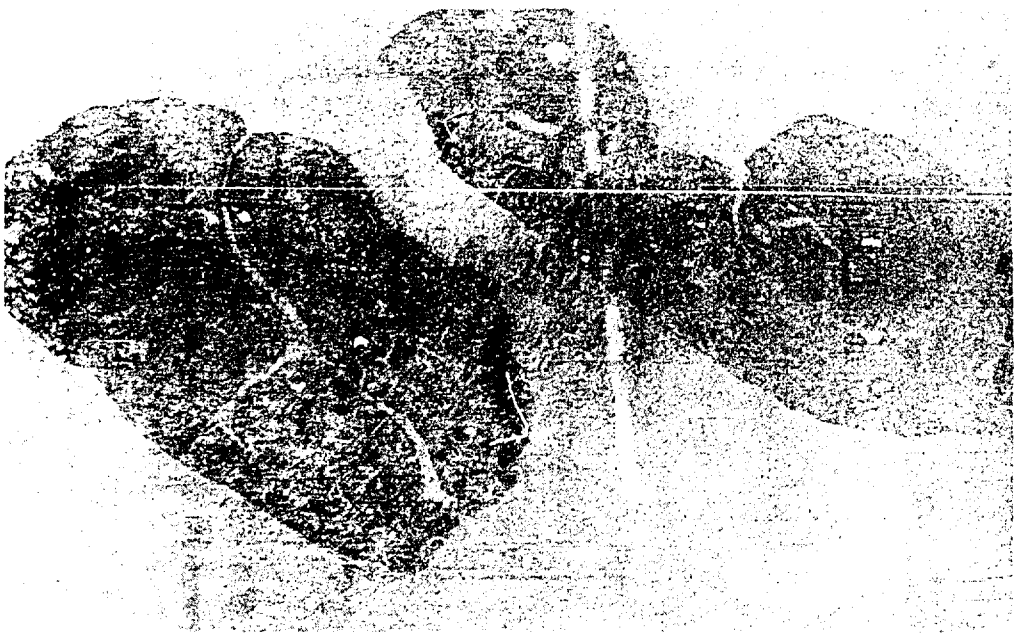

Figure 5. Slide Section, Lung Moderately Affected (1.5X).

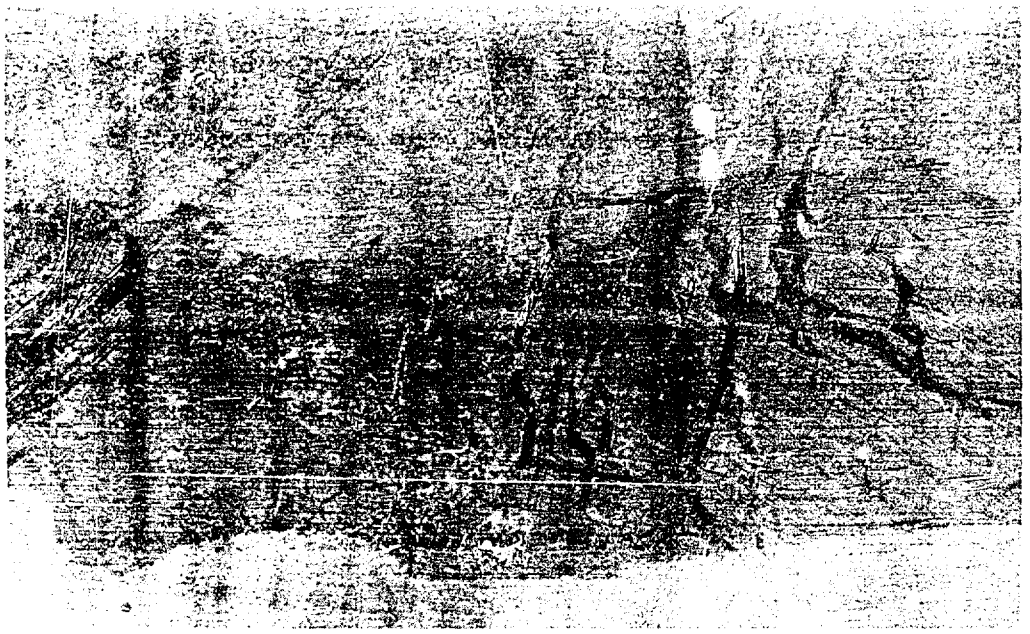

Figure 6. Slide Section, Lung Severely Affected (1.5X).

These values remained well within normal limits. Although blood serum levels of the drug were not determined in our dogs, the absence of renal damage is attributed to the small amount absorbed into the blood stream from the digestive tract.

The number of dogs used in this study is admittedly small. However, eight of twelve dogs subcutaneously vaccinated and subsequently treated were negative to severe aerosol challenge. The remaining four responded very minimally.

V. CONCLUSIONS

Two conclusions were reached as a result of these studies:

(a) Effective immunization via the pulmonary route with killed organisms did not occur, possibly because of the efficiency of the lung clearance mechanism.

(b) The incidence and severity of coccidioidomycosis in aerosol-challenged dogs can be safely and materially reduced by combining the physiological effects of a subcutaneously administered killed arthrospore vaccine with daily, orally administered, presolubilized Amphotericin B.

LITERATURE CITED

1. Negroni, P.; Vivoli, D.; and Bonfiglioli, H. "Estudios sobre el Coccidioides immitis Rixford et Gilchrist: VII. Reacciones immunoalergicas en la infeccion experimental del cobayo," Rev. Inst. Malbran (Buenos Aires) 14:273-286, 1949.

2. Vogel, R.A.; Fetter, B.F.; Conant, N.R.; and Lowe, E.P. "Preliminary studies on artificial active immunization of guinea pigs against respiratory challenge with Coccidioides immitis," Am. Rev. Tuberc. 79:498-503, 1954.

3. Friedman, L., and Smith, C.E. "Vaccination of mice against Coccidioides immitis," Am. Rev. Tuberc. Pulmonary Diseases 74:245-248, 1956.

4. Levine, H.B.; Cobb, J.M.; and Smith, C.E. "Immunity of coccidioidomycosis introduced in mice by purified spherule, arthrospore, and mycelial vaccines," Trans. N. Y. Acad. Sci. 22:436-449, 1960.

5. Levine, H.B.; Cobb, J.M.; and Smith, C.E. "Immunogenicity of spherule-endospore vaccines of Coccidioides immitis for mice," J. Immunol. 87:218-227, 1961.

6. Converse, J.L.; Castleberry, M.W.; Besemer, A.R.; and Snyder, E.M. "Immunization of mice against coccidioidomycosis," J. Bacteriol. 84:46-52, 1962.

7. Kong, Yi-Chi M.; Levine, H.B.; and Smith, C.E. "Immunogenic properties of nondisrupted and disrupted spherules of Coccidioides immitis in mice," Sabouraudia 2:131-142, 1963.

8. Levine, H.B.; Miller, R.L.; and Smith, C.E. "Influence of vaccination on respiratory coccidioidal disease in cynomolgus monkeys," J. Immunol. 89:242-251, 1962.

9. Campbell, C.C., and Hill, G.B. "Beneficial therapeutic effects of solubilized Amphotericin B following oral administration in experimental coccidioidomycosis, histoplasmosis and crytococcosis in mice," Transactions, Fourth Annual Meeting VA-AF Coccidioidomycosis Cooperative Study, 1959. pp. 20-21

10. Wolfe, E.K., Jr. "Quantitative characterization of aerosols," Bact. Rev. 25:194-202, 1961.

11. Hitchner, S.B., and Reising, G. "Flock vaccination for Newcastle disease by atomization of the B_1 strain of virus," Proc. Am. Vet. Med. Assn., 89th Annual Meeting, 1952. p. 258

12. Aleksandrov, N.I.; Gefen, N.Ye.; Garin, N.S.; Gapochko, K.G.; Daal-Berg, I.I.; and Sergeyer, V.M. "Reactogenicity and effectiveness of aerogenic vaccination against certain zoonoses," Voyenno-Meditsinskiy Zhurnal 12:34-38, 1958.

13. Eigelsbach, H.T.; Tulis, J.J.; Overholt, E.L.; and Griffith, W.R. "Aerogenic immunization of the monkey and guinea pig with live tularemia vaccine," Proc. Soc. Exptl. Biol. Med. 108:732-734, 1961.

14. Bell, N.H.; Andriole, V.T.; Sabesin, S.M.; and Utz, J.P. "Nephrotoxicity of Amphotericin B," Amer. J. Med. 33:64-69, 1962.

15. Sanford, W.G.; Rasch, J.R.; and Stonehill, R.B. "A therapeutic dilemma," Ann. Internal Med. 56:553-563, 1962.